CHIMERIC PEPTIDES AS IMMUNOGENS, ANTIBODIES THERETO, AND METHODS FOR IMMUNIZATION USING CHIMERIC PEPTIDES OR ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

5   The present application claims the benefit of priority under 35 U.S.C. §119(e) from U.S. provisional application no. 60/169,687, filed December 8, 1999, the entire contents of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

10   The present invention relates to a chimeric peptide immunogen containing a B cell epitope joined to a T cell epitope from a different source, immunizing compositions containing the chimeric peptide, and a method for immunization using same.

Description of the Background Art

15   A major histopathological hallmark of Alzheimer's Disease (AD) is the presence of amyloid deposits within neuritic and diffuse plaques in the parenchyma of the amygdala, hippocampus and neocortex (Glenner and Wong, 1984; Masters et al., 1985; Sisodia and Price, 1995). Amyloid is a generic term

20   that describes fibrillar aggregates that have a common β-pleated structure. These aggregates exhibit birefringent properties in the presence of Congo red and polarized light (Glenner and Wong, 1984). The diffuse plaque is thought to be relatively benign in

contrast to the neuritic plaque which appears to be strongly correlated with reactive and degenerative processes (Dickson et al., 1988; Tagliavini et al., 1988; Yamaguchi et al., 1989; Yamaguchi et al., 1992). The principal component of neuritic plaques is a 42 amino acid residue amyloid-β (Aβ) peptide (Miller et al., 1993; Roher et al., 1993) that is derived from the much larger β-amyloid precursor protein, βAPP (or APP) (Kang et al., 1987). Two major C-terminal variants of amyloid-β peptide, Aβ 1-40 ending at Val40 and Aβ 1-42(43) ending at Ala42 or Thr43, proteolytically cleaved from βAPP, were found in amyloid deposits (Miller et al., 1993; Roher et al., 1993). Aβ 1-42 is produced less abundantly than the 1-40 Aβ peptide (Haass et al., 1992; Seubert et al., 1992), but the preferential deposition of Aβ1-42 results from the fact that this COOH-extended form is more insoluble than 1-40 Aβ and is more prone to aggregate and form anti-parallel β-pleated sheets (Joachim et al., 1989; Halverson et al., 1990; Barrow et al., 1992; Burdick et al., 1992; Fabian et al., 1994). Aβ1-42 can seed the aggregation of Aβ 1-40 (Jarrett and Lansbury 1993). Iwatsubo et al., (1996) and Saido et al., (1996) further reported that other variant amyloid-β peptides, Aβ 3(pyroglutamate)-42, Aβ 11(pyroglutamate)-42, Aβ 17-42, Aβ 1(D-Asp)-42, and Aβ 1(L-isoAsp)-42 were also found to be present in amyloid deposits in the brain.

The APP gene was sequenced and found to be encoded on chromosome 21 (Kang et al., 1987). Expression of the APP gene generates several Aβ-containing isoforms of 695, 751 and 770

amino acids (Figure 1), with the latter two βAPP containing a

domain that shares structural and functional homologies with

Kunitz serine protease inhibitors (Kang et al., 1987; Kitaguchi

et al., 1988; Ponte et al., 1988; Tanzi et al., 1988; Konig et

5   al., 1992). The functions of βAPP in the nervous system remain

to be defined, although there is increasing evidence that βAPP

has a role in mediating adhesion and growth of neurons (Schubert

et al., 1989; Saitoh et al., 1994; Saitoh and Roch, 1995) and

possibly in a G protein-linked signal transduction pathway

10   (Nishimoto et al., 1993). In cultured cells, βAPPs mature

through the constitutive secretory pathway (Weidemann et al.,

1989; Haass et al., 1992; Sisodia 1992) and some cell-surface-

bound βAPPs are cleaved within the Aβ domain by an enzyme,

designated α-secretase, (Esch et al., 1990), an event that

15   precludes Aβ amyloidogenesis (Figure 1). Several studies have

delineated two additional pathways of βAPP processing that are

both amyloidogenic: first an endosomal/lysosomal pathway

generates a complex set of βAPP-related membrane-bound fragments,

some of which contain the entire Aβ sequence (Haass et al., 1992;

20   Golde et al., 1992); and second, by mechanisms that are not fully

understood, Aβ 1-40 is secreted into the conditioned medium and

is present in cerebrospinal fluid in vivo (Haass et al., 1992;

Seubert et al., 1992; Shoji et al., 1992; Busciglio et al.,

1993). Lysosomal degradation is no longer thought to contribute

25   significantly to the production of Aβ (Sisodia and Price, 1995).

The proteolytic enzymes responsible for the cleavages at the NH_2

and COOH termini of Aβ termed β and γ (Figure 1), respectively, have not been identified. Until recently, it was generally believed that Aβ is generated by aberrant metabolism of the precursor. The presence, however, of Aβ in conditioned medium of

5   a wide variety of cells in culture and in human cerebrospinal fluid indicate that Aβ is produced as a normal function of cells.

If amyloid deposition is a rate-limiting factor to produce AD, then all factors linked to the disease will either promote amyloid deposition or enhance the pathology that is

10 provoked by amyloid. The likelihood of amyloid deposition is enhanced by trisonomy 21 (Down's syndrome) (Neve et al., 1988; Rumble et al., 1989), where there is an extra copy of the APP gene, by increased expression of APP, and by familial Alzheimer's Disease (FAD)-linked mutations (Van Broeckhoven et al., 1987;

15 Chartier-Harlin et al., 1991; Goate et al., 1989; Goate et al., 1991; Murrell et al., 1991; Pericak-Vance et al., 1991; Schellenberg et al., 1992; Tanzi et al., 1992; Hendricks et al., 1992; Mullan et al., 1992). Some of these mutations are correlated with an increased total production of Aβ (Cai et al.,

20 1993; Citron et al., 1992) or specific overproduction of the more fibrillogenic peptides (Wisniewski et al., 1991; Clements et al., 1993; Suzuki et al., 1994) or increased expression of factors that induce fibril formation (Ma et al., 1994; Wisniewski et al., 1994). Collectively, these findings strongly favor the

25 hypothesis that amyloid deposition is a critical element in the development of AD (Hardy 1992), but of course they do not

preclude the possibility that other age-related changes associated with the disease, such as paired helical filaments, may develop in parallel rather than as a result of amyloid deposition and contribute to dementia independently.

5   The main focus of researchers and the principal aim of those associated with drug development for AD is to reduce the amount of Aβ deposits in the central nervous system (CNS). These activities fall into two general areas: factors affecting the production of Aβ, and factors affecting the formation of insoluble Aβ fibrils. A third therapeutic goal is to reduce inflammatory responses evoked by Aβ neurotoxicity.

          With regards to the first, a major effort is underway to obtain a detailed understanding of how newly synthesized βAPP is processed for insertion into the plasma membrane and to identify the putative amyloidogenic secretases that have been assigned on the basis of sites for cleavage in the mature protein. From a pharmacological perspective, the most direct way of reducing the production of Aβ is through direct inhibition of either β or γ secretase. No specific inhibitors are currently available although a number of compounds have been shown to indirectly inhibit the activities. Bafilomycin, for example, inhibits Aβ production with an EC_{50} of about 50 nM (Knops et al., 1995; Haass et al., 1995), most likely through its action as an inhibitor of vascular $H^{+}ATPase$ co-localized in vesicles with the Aβ secretase. Another compound, MDL28170, used at high concentrations appears to block the activity of γ secretase

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Higaki et al., 1995). It is generally hoped that the identification of the β or γ secretases might lead to the synthesis of specific protease inhibitors to block the formation of amyloidogenic peptides. It is not known, however, whether

5 these enzymes are specific for βAPP or whether they perhaps have other important secretory functions.

Similarly, problems of target and targeting specificity will be encountered through any attempt to interfere with signal transduction pathways that may determine whether processing of

10 βAPP is directed through the amyloidogenic or non-amyloidogenic pathways. Moreover, these signal transduction mechanisms still need to be identified. In conclusion, present understanding of the complex and varied underlying molecular mechanisms leading to overproduction of Aβ offers little hope for selective targeting

15 by pharmacological agents.

Given that neurotoxicity appears to be associated with β-pleated aggregates of Aβ, one therapeutic approach is to inhibit or retard Aβ aggregation. The advantage of this approach is that the intracellular mechanisms triggering the

20 overproduction of Aβ or the effects induced intracellularly by Aβ need not be well understood. Various agents that bind to Aβ are capable of inhibiting Aβ neurotoxicity *in vitro,* for example, the Aβ-binding dye, Congo Red, completely inhibits Aβ-induced toxicity in cultured neurons (Yankner et al., 1995). Similarly,

25 the antibiotic rifampacin also prevents Aβ aggregation and subsequent neurotoxicity (Tomiyama et al., 1994). Other

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compounds are under development as inhibitors of this process
either by binding Aβ directly, e.g., hexadecyl-N-
methylpiperidinium (HMP) bromide (Wood et al., 1996), or by
preventing the interaction of Aβ with other molecules that
5   contribute to the formation of Aβ deposition. An example of such
a molecule is heparan sulfate or the heparan sulfate
proteoglycan, perlecan, which has been identified in all amyloids
and is implicated in the earliest stages of inflammation
associated amyloid induction.

10   Heparan sulfate interacts with the Aβ peptide and
imparts characteristic secondary and tertiary amyloid structural
features. Recently, small molecule anionic sulfates have been
shown to interfere with this reaction to prevent or arrest
amyloidogenesis (Kisilevsky, 1995), although it is not evident
15   whether these compounds will enter the CNS. A peptide based on
the sequence of the perlecan-binding domain appears to inhibit
the interaction between Aβ and perlecan, and the ability of Aβ-
derived peptides to inhibit self-polymerization is being explored
as a potential lead to developing therapeutic treatments for AD.
20   The effectiveness of these compounds *in vitro*, however, is likely
to be modest for a number of reasons, most notably the need for
chronic penetration of the blood brain barrier.

         As another means of inhibiting or retarding Aβ
aggregation, WO 96/25435 discloses the potential for using a
25   monoclonal antibody, which is end-specific for the free C-
terminus of the Aβ 1-42 peptide, but not for the Aβ 1-43 peptide,

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in preventing the aggregation of Aβ 1-42. While the administration of such an Aβ end-specific monoclonal antibody is further disclosed to interact with the free C-terminal residue of Aβ 1-42, thereby interfering with and disrupting aggregation that may be pathogenic in AD, there is no specific disclosure on how these Aβ C-terminal-specific monoclonal antibodies would be used therapeutically. Although direct or indirect manipulation of Aβ peptide aggregation appears to be an attractive therapeutic strategy, a possible disadvantage of this general approach may be that pharmacological compounds of this class will need to be administered over a long period of time, and may accumulate and become highly toxic in the brain tissue.

WO 98/44955 takes a novel approach to avoiding the problems associated with repeated administration of pharmacological agent and discloses a method for preventing the onset of Alzheimer's Disease or for inhibiting progression of Alzheimer's Disease through the stable ectopic expression in the brain of recombinant antibodies end-specific for amyloid-β peptides.

Recently, Schenk et al. (1999) demonstrated that immunization with amyloid-β attenuated Alzheimer's disease-like pathology in PDAPP transgenic mice serving as an animal model for amyloid-β deposition and Alzheimer's disease-like neuropathologies. They reported that immunization of young animals prior to the onset of Alzheimer's disease-type neuropathologies essentially prevented the development of β-

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amyloid plaque formation, neuritic dystrophy and astragliosis, whereas treatment in older animals after the onset of Alzheimer's disease-type neuropathologies was observed to reduce the extent and progression of these neuropathologies.

Although the results reported by Schenk et al. provides promise for using immunomodulation as a general approach to treat Alzheimer's disease, immunization with intact amyloid-β according to Schenk et al. has several problems that need to be addressed in developing an immunization program for treatment of Alzheimer's disease in humans. One problem is that it is not clear how readily one can raise an anti-self antibody response by immunizing humans with human amyloid-β. Moreover, even if an anti-self antibody response is raised against human amyloid-β, it is unclear whether or not auto-immunity might develop which would be injurious to the patient. Other problems include how the immunization would be reversed or halted as the antigen in the form of endogenous amyloid-β is always available to the patients' immune system and whether or not administering amyloid-β, which is believed to be neurotoxic, would have severe and adverse pharmacological effects on the patient.

An alternative to a peptide-based approach is to elucidate the cellular mechanism of Aβ neurotoxicity and develop therapeutics aimed at those cellular targets. The focus has been on controlling calcium dysfunction of free radical mediated neuronal damage. It has been postulated that Aβ binds to RAGE (the receptor for advanced glycation end-products) on the cell

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surface, thereby triggering reactions that could generate
cytotoxic oxidizing stimuli (Yan et al., 1996). Blocking access
of Aβ to the cell surface binding site(s) might retard
progression of neuronal damage in AD. To date there are no
5   specific pharmacological agents for blocking Aβ-induced
neurotoxicity.

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10   present application. Any statement as to content or a date of
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the correctness of such a statement.

SUMMARY OF THE INVENTION

15   The present invention provides a chimeric peptide or
mixture of chimeric peptides with an end-specific B cell epitope
from a naturally-occurring internal peptide cleavage product of a
precursor or mature protein, as free N-terminus or C-terminus,
fused with or without spacer amino acid residue(s) to a T helper
20   cell epitope derived from a source different from that of the
internal peptide cleavage product.

The present invention also provides an immunizing
composition which includes an immunizing effective amount of the
chimeric peptide or mixture of chimeric peptides. This
25   immunizing composition is used to raise antibodies in a subject

mammal where the internal peptide cleavage product, for which the B cell epitope of the chimeric peptide is end-specific, is a self molecule.

Further provided by the present invention is a method for immunization of a mammal against the free N-terminus or free C-terminus of an internal self peptide cleavage product derived from a self precursor or mature protein. More particularly, the method for immunization is directed to raising antibodies and immunizing individuals against amyloid β peptides associated with amyloid β deposits and plaques.

Yet other aspects of the present invention are directed to a molecule which includes the antigen-binding portion of an antibody specific for the chimeric peptide according to the present invention and a method for passive immunization using this molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic representation of the β-amyloid precursor protein (βAPP) and the products of α, β, γ-secretase cleavage. The general locations of various domains are indicated along with the cleavage sites for α, β, and γ-secretases.

Figure 2 shows a partial amino acid sequence (SEQ ID NO:1) of the region of βAPP from which amyloid-β peptides (Aβ) are derived. The arrows indicate the α-, β-, and γ- secretase cleavage sites.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

One aspect of the present invention is directed generally to a chimeric peptide or a mixture of chimeric peptides in which a N- or C-terminal end-specific B cell epitope from a

5 naturally-occurring internal peptide cleavage product of a precursor or mature protein, as a free N- or C-terminus, is fused with or without spacer amino acid residue(s) to a T helper cell epitope from a different source. The chimeric peptide or peptides are used in an immunizing composition for immunizing a

10 mammal against the free N-terminus or free C-terminus of an internal peptide cleavage product which is a self molecule of the immunized mammal (molecule native thereto). More specifically as a preferred embodiment of the present invention, the chimeric peptide(s) have an N- or C-terminal end-specific B cell epitope,

15 which is the first two to five amino acid residues of the N-terminus or the last two to five amino acid residues of the C-terminus of an amyloid β peptide, fused to a T helper cell epitope. When such chimeric peptide(s) are administered to a human individual as part of an immunizing composition, that

20 individual will be immunized against the amyloid β peptide or peptides from which the end-specific B cell epitope is derived.

By way of the preferred embodiments of a chimeric peptide containing an amyloid β peptide-specific B cell epitope and a method for immunizing against an amyloid β peptide as

25 examples of the present invention, those of skill in the art will readily appreciate that the chimeric peptides(s), immunizing

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composition, and method for immunization can be extended and
directed to other internal peptide cleavage products, for which
end-specific antibodies raised by the present method would not be
cross-reactive with the precursor or mature self protein.

5   Similarly, most of the advantages described below for the method
for immunization according to the present invention, which raises
end-specific antibodies against amyloid β peptides in humans as a
preferred embodiment, are generally applicable for immunization
with the chimeric peptide or peptides of the present invention.

10   It is well-known that antibody responses produced by B
cells to a defined region of a protein or peptide require that T
helper cells of the immune system recognize another part of that
antigen simultaneously. This is commonly referred to as B/T
cell collaboration. According to the present invention, this

15   phenomena can be mimicked by making a synthetic chimeric peptide
which contains both B and T cell epitopes in a contiguous linear
sequence. Such chimeric peptides have been used very
successfully to drive antibody production in mice, human/mice
chimeras and primates (Sharma et al., 1993; Ifversen et al.,

20   1995; O'Hern et al., 1997).

          In the present invention, the B cell epitope containing
the first two to five amino acid residues of the free N-terminus
or the last two to five amino acid residues of the free C-
terminus of an amyloid β peptide is fused, with or without spacer

25   amino acid residues, to a known strong T helper cell epitope to
form a chimeric peptide. A non-limiting example of such a known

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strong T cell epitope is the well-studied tetanus toxoid promiscuous epitope of SEQ ID NO:8 (Ho et al, 1990; Panina-Bordignon et al., 1989) as this epitope is known to work in a number of diverse human genetic backgrounds (Valmori et al., 1992

5   and 1994).

Immunization with the chimeric peptides(s) containing an amyloid β end-specific B cell epitope and a promiscuous T helper cell epitope of tetanus toxoid, as a preferred embodiment, should give rise to antibodies with the following specificities:

10   (1) anti-tetanus antibody, which would be irrelevant in humans as most individuals are already sera-positive for tetanus toxoid (i.e., from previous tetanus immunizations);

(2) anti-junction antibodies, which recognize novel epitopes created by the junction joining the end-specific B-cell

15   epitope of an amyloid β peptide and a T helper cell epitope of tetanus toxoid, but would not recognize anything other than the immunogen itself, and therefore would be irrelevant *in vivo* in the immunized individual; and

(3) anti-N-terminal or anti-C-terminal end-specific

20   amyloid β antibodies, which are the desired antibodies sought to be raised by the method according to the present invention for inhibiting, reducing, or even perhaps reversing amyloid β deposit/plaque formation.

The desired anti-N-terminal or anti-C-terminal end-

25   specific amyloid β antibodies raised by the method for immunization according to the present invention are able to

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discriminate between an amyloid β peptide and the β amyloid
protein precursor (βAPP) from which it is proteolytically
derived. These end-specific amyloid β antibodies bind
specifically to the terminus/end of an amyloid β peptide to slow

5   down, reduce or prevent the accumulation of amyloid β peptides in
the extracellular space, interstitial fluid and cerebrospinal
fluid of the brain, and the aggregation into senile amyloid
deposits or plaques and to block the interaction of amyloid β
peptides with other molecules that contribute to the

10 neurotoxicity of amyloid β.

     The presence of anti-N-terminal or anti-C-terminal end-
specific amyloid β antibodies in the blood and in the
extracellular space, interstitial fluid and cerebrospinal fluid
of the brain, where soluble amyloid β peptides are present,

15 promotes the formation of soluble antibody-amyloid β complexes.
These soluble antibody-amyloid β complexes are cleared from the
central nervous system by drainage of the extracellular space,
interstitial fluid and cerebrospinal fluid into the general blood
circulation through the arachnoid villi of the superior sagittal

20 sinus. In this manner, soluble amyloid β peptides are prevented
from accumulating in the extracellular space, interstitial fluid
and cerebrospinal fluid to form amyloid deposits and/or to induce
neurotoxicity (Fig. 1). Furthermore, clearance of soluble
amyloid-β peptides in accordance with the method of the present

25 invention is expected to reduce the inflammatory process observed
in Alzheimer's Disease by inhibiting, for example, amyloid-β-

induced complement activation and cytokine release, and by also blocking the interaction of Aβ with cell surface receptors such as the RAGE receptor.

As shown in Fig. 1 (see Schehr, 1994), and discussed in the Background Art section, the β-amyloid protein precursor (βAPP) is believed to also serve as a precursor for a proteolytic product, soluble β-amyloid protein precursor (sβAPP), thought to have growth promoting and neuroprotective functions. It will be readily appreciated by those of skill in the art that the anti-N-terminal or anti-C-terminal end-specific amyloid β antibodies will not interfere with the normal biological functions of βAPP that are not associated with the formation of amyloid β peptides.

The advantages of the method for immunization according to the preferred embodiment of the present invention include:

(1) a cheap peptide antigen, that is readily and easily produced and controlled for quality assurance, is used in active immunization;

(2) inclusion of only two to three, and perhaps up to four or five, amino acid residues from the N- or C- terminus of an internal peptide cleavage product (amyloid β peptide) should minimize the amount of antibody produced which reacts with the precursor or mature protein from which the internal peptide cleavage product is proteolytically derived (βAPP), and hence might interfere with the function of this precursor or mature protein;

(3) use of an independent non-self T cell epitope should break self-tolerance and allow production of antibodies to a self-antigen (Schofield et al., 1991);

(4) the absence in the chimeric peptide(s) of a T cell epitope from the internal peptide cleavage product (amyloid β) should avoid any significant problems of autoimmunity, since anti-self T cell immunity underlies progression of all known autoimmune diseases; and

(5) the immunization should be self-limiting and reversible, with antibody titers gradually falling off with time, since the patient's immune system will never naturally encounter the combination of amyloid β with tetanus toxin as immunogen. This self-limiting and reversible immunization has been demonstrated in clinical trials with synthetic contraceptive vaccines (Talwar et al., 1997).

The chimeric peptide of the present invention is represented by formula (I) or formula (II)

(I) $N-(S)_m-(T_h)_n$

(II) $(T_h)_n-(S)_m-C$
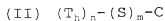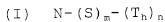
where:

N is the first 2, 3, 4 or 5 amino acid residues from the free N-terminus of a naturally-occurring internal peptide cleavage product, such as an amyloid β peptide, which, when naturally-occurring in a mammal, is derived from a precursor protein or a mature protein;

C is the last 2, 3, 4 or 5 amino acid residues from the free C-terminus of the naturally occurring internal peptide cleavage product;

T_h is a T helper cell epitope derived from a natural source (i.e., species of living organism) different from that of the naturally-occurring internal peptide cleavage product

S is a spacer amino acid residue;

m is 0, 1, 2, 3, 4, or 5; and

n is 1, 2, 3, or 4.

Also contemplated are mixtures of chimeric peptides of formula (I) and formula (II) in which the internal peptide cleavage product of each chimeric peptide can be the same or different, i.e., different amyloid β peptides. These chimeric peptide mixtures are however not limited to mixtures of formula (I) and formula (II) chimeric peptides and can include mixtures of formula (I) peptides, where N is from different internal peptide cleavage products (i.e., different amyloid β peptides), or mixtures of formula (II) peptides, where C is from different internal peptide cleavage products (i.e., different amyloid β peptides).

The chimeric peptide of the present invention has a length between about 20 to 90 amino acid residues, preferably between about 20 to 75 amino acid residues, more preferably between about 20 to 50 amino acid residues, and most preferably between about 20 to 40 amino acid residues.

The B cell epitope from the free N-terminus (N) or the free C-terminus (C) of the naturally-occurring internal peptide cleavage product is preferably two or three amino acid residues, which is not sufficient for raising antibodies cross-reactive

5   with the precursor protein or the mature protein of the peptide cleavage product. A B cell epitope of four or five amino acid residues from the free N-terminus or the free C-terminus of the naturally-occurring internal peptide cleavage product may also be suitable, although there is a slightly increased possibility that

10   some antibodies may be raised which are cross-reactive with the precursor protein or the mature protein.

A preferred chimeric peptide embodiment of the present invention has a N or C in formula (I) or (II), respectively, from an amyloid β peptide which, when naturally-occurring in humans,

15   is derived from βAPP. As used herein, the term "derived from" means that, when obtained from a natural source, the peptide or protein is the result of cleavage or conversion from a precursor protein or a mature protein, whether by a single proteolytic cleavage event or by more than one cleavage and/or conversion

20   event. In the case of the preferred embodiment, any amyloid β peptide found to occur naturally in amyloid deposits, fibrils, and plaques is encompassed by the term "amyloid β peptide" as the internal peptide cleavage product. Peptides of SEQ ID NOs:2 (Aβ1-40), 3 (Aβ1-42), 4 (Aβ1-43), 5 (Aβ3-42), 6 (Aβ11-42), and 7

25   (Aβ17-42) are non-limiting examples of such "amyloid β peptides".

T_h is a T helper cell epitope that is derived from a
natural source different from the source of the naturally-
occurring internal peptide cleavage product. In other words, the
T helper cell epitope is not recognized as part of a self-
molecule in the mammal subject immunized according to the method
of the present invention. The T helper cell epitope is combined
with a B cell epitope specific to the N-terminus or C-terminus of
peptide cleavage product which is internal (not located at the
ends) to a precursor or mature protein to evoke an efficient
antibody response.

It is known that immunogens must be presented in
conjunction with major histocompatibility (MHC) class II antigens
to evoke an efficient antibody response. The MHC class II
antigens produced by antigen-presenting cells (APCs) bind to T
cell epitopes present in the immunogen in a sequence specific
manner. This MHC class II-immunogen complex is recognized by
CD4$^+$ lymphocytes (T_h cells), which cause the proliferation of
specific B cells capable of recognizing a B cell epitope from the
presented immunogen and the production of B cell epitope-specific
antibody by such B cells. Since amyloid β peptides are self
molecules, they do not possess any recognizable T_h epitopes, and
B cell epitopes of 2 to 5 amino acid residues would lack any T
cell epitopes altogether. Such epitopes can be provided by
specific sequences derived from potent immunogens including
tetanus toxin, pertussis toxin, the measles virus F protein and
the hepatitis B virus surface antigen (HBsAg). The T_h epitopes

selected are preferably capable of eliciting T helper cell
responses in large numbers of individuals expressing diverse MHC
haplotypes. These epitopes function in many different
individuals of a heterogeneous population and are considered to

5   be promiscuous T_h epitopes. Promiscuous T_h epitopes provide an
advantage of eliciting potent antibody responses in most members
of genetically diverse population groups.

     Moreover, the T helper cell epitopes in the chimeric
peptide of the present invention are also advantageously selected

10   not only for a capacity to cause immune responses in most members
of a given population, but also for a capacity to cause
memory/recall responses. When the mammal is human, the vast
majority of human subjects/patients receiving immunotherapy with
the chimeric peptide of the present invention will already have

15   been immunized with the pediatric vaccines (i.e.,
measles+mumps+rubella and diphtheria+pertussis+tetanus vaccines)
and, possibly, the hepatitis B virus vaccine. These patients
have therefore been previously exposed to at least one of the T_h
epitopes present in chimeric pediatric vaccines. Prior exposure

20   to a T_h epitope through immunization with the standard vaccines
should establish T_h cell clones which can immediately proliferate
upon administration of the chimeric peptide (i.e., a recall
response), thereby stimulating rapid B cell responses to the
chimeric peptide. In addition, the T_h epitopes avoid any

25   pathogen-specific B cell and/or suppressor T cell epitopes which
could lead to carrier-induced immune suppression, a problem

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encountered when toxin molecules are used to elicit T helper cell responses.

The T_h epitopes in the chimeric peptide of the invention are promiscuous but not universal. This characteristic means that the T_h epitopes are reactive in a large segment of an outbred population expressing different MHC antigens (reactive in 50 to 90% of the population), but not in all members of that population. To provide a comprehensive, approaching universal, immune reactivity for an internal peptide cleavage product, a combination of chimeric peptides with different T_h epitopes can be prepared. For example, a combination of four chimeric peptides with promiscuous T_h epitopes from tetanus and pertussis toxins, measles virus F protein and HBsAg may be more effective.

Promiscuous T_h epitopes often share common structural features. For example, promiscuous T_h epitopes range in size from about 15 to about 30 residues. Amphipathic helices are a common feature of the T_h epitopes. An amphipathic helix is defined by an α-helical structure with hydrophobic amino acid residues dominating the surrounding faces. T_h epitopes frequently contain additional primary amino acid patterns such as a Gly or a charged reside followed by two to three hydrophobic residues followed in turn by a charged or polar residue. This pattern defines Rothbard sequences. T_h epitopes often obey the 1, 4, 5, 8 rule, where a positively charged residue is followed by hydrophobic residues at the fourth, fifth and eighth positions after the charged residue. Since all of these structures are

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composed of common hydrophobic, charged and polar amino acids, each structure can exist simultaneously within a single T_h epitope.

T_h is therefore a sequence of amino acids (natural or non-natural) that contain a T_h epitope. A T_h epitope can be a continuous or discontinous epitope. Hence, not every amino acid of T_h is necessarily part of the epitope. Accordingly, T_h epitopes, including analogs and segments of T_h epitopes, are capable of enhancing or stimulating an immune response to the internal peptide cleavage product. Immunodominant T_h epitopes are broadly reactive in animal and human populations with widely divergent MHC types (Celis et al., 1988; Demotz et al., 1989; and Chong et al., 1992). The T_h domain of the chimeric peptides of the present invention has from about 10 to about 50 amino acids residues and preferably from about 10 to about 30 amino acids residues. When multiple T_h epitopes are present (i.e., $n \geq 2$), then each T_h epitope is independently the same or different.

T_h epitope analogs include substitutions, deletions and insertions of one to about five amino acid residues in the T_h epitope. T_h segments are contiguous portions of a T_h epitope that are sufficient to enhance or stimulate an immune response to the internal peptide cleavage product. An example of T_h segments is a series of overlapping peptides that are derived from a single longer peptide.

The T_h epitopes of the present invention include hepatitis B surface antigen T helper cell epitopes (HB_s T_h),

pertussis toxin T helper cell epitopes (PT T_h), tetanus toxin T helper cell epitopes (TT T_h), measles virus F protein T helper cell epitope (MV_{F1} T_h), *Chlamydia trachomitis* major outer membrane protein T helper cell epitopes (CT T_h), diphtheria toxin T helper cell epitopes (DT T_h), *Plasmodium falciparum* circumsporozoite T helper cell epitopes (PF T_h), *Schistosoma mansoni* triose phosphate isomerase T helper cell epitopes (SM T_h), *Escherichia coli* TraT T helper cell epitopes (TraT T_h) and immune-enhancing analogs and epitope sequences are provided below in Table 1.

TABLE 1

TT_o T_h	SEQ ID NO:8
HB_3 T_h	SEQ ID NO:9
TT_1 T_h	SEQ ID NO:10
TT_1 T_h	SEQ ID NO:11
TT_2 T_h	SEQ ID NO:12
PT_{1A} T_h	SEQ ID NO:13
TT_3 T_h	SEQ ID NO:14
PT_2 T_h	SEQ ID NO:15
MV_{F1} T_h	SEQ ID NO:16
MV_{F2} T_h	SEQ ID NO:17
TT_4 T_h	SEQ ID NO:18
TT_5 T_h	SEQ ID NO:19
CT_2 T_h	SEQ ID NO:20
DT_1 T_h	SEQ ID NO:21
DT_2 T_h	SEQ ID NO:22
PF T_h	SEQ ID NO:23
SM T_h	SEQ ID NO:24
$TraT_1$ T_h	SEQ ID NO:25
$TraT_2$ T_h	SEQ ID NO:26
$TraT_3$ T_h	SEQ ID NO:27

Immunogenicity can be improved through the addition of
spacer residue(s) S (i.e. Gly-Gly) between the promiscuous T_h
epitope and the B cell epitope of the chimeric peptide according
to the present invention. In addition to physically separating
the T_h epitope from the B cell epitope, the glycine spacer
residues can disrupt any artificial secondary structures created

by the joining of the T_h epitope with the B cell epitope, and thereby eliminate interference between the T and/or B cell responses. The conformational separation between the helper epitope and the antibody eliciting domain thus permits more

5   efficient interactions between the presented immunogen and the appropriate T_h and B cells. The amino acid residue(s) for S can be naturally-occurring amino acids or non-naturally-occurring amino acids, which include, but are not limited to, β-alanine, ornithine, norleucine, norvaline, hydroxyproline, thyroxine,

10 gamma-amino butyric acid, homoserine, citrulline and the like.

The chimeric peptides of the present invention can be made by synthetic chemical methods which are well known to the ordinarily skilled artisan. Hence, the chimeric peptides can be synthesized using the automated Merrifield techniques of solid

15 phase synthesis with either t-Boc or F-moc chemistry on Peptide Synthesizers such as an Applied Biosystems Peptide Synthesizer.

After complete assembly of the desired chimeric peptide, the resin is treated according to standard procedures to cleave the peptide from the resin and deblock the protecting

20 groups on the amino acid side chains. The free peptide is purified by HPLC and characterized biochemically, for example, by amino acid analysis or by sequencing. Purification and characterization methods for peptides are well-known to one of ordinary skill in the art.

25 Alternatively, the longer linear chimeric peptides can be synthesized by well-known recombinant DNA techniques. Any

standard manual on DNA technology provides detailed protocols to
produce the chimeric peptides of the invention. To construct a
gene encoding a chimeric peptide of the present invention, the
amino acid sequence is reverse transcribed into a nucleic acid
5   sequence, and preferably using optimized codon usage for the
organism in which the gene will be expressed. Next, a synthetic
gene is made, typically by synthesizing overlapping
oligonucleotides which encode the peptide and any regulatory
elements, if necessary. The synthetic gene is inserted in a
10   suitable cloning vector and recombinant clones are obtained and
characterized. The chimeric peptide is then expressed under
suitable conditions appropriate for the selected expression
system and host, and the chimeric peptide is purified and
characterized by standard methods.

15   As an optional segment to the chimeric peptide, an
immunostimulatory epitope of the invasin protein of a *Yersinia*
species can be linked to the T helper cell epitope of the
chimeric peptide opposite from the B cell epitope. The invasins
of the pathogenic bacteria Yersinia spp. are outer membrane
20   proteins which mediate entry of the bacteria into mammalian cells
(Isberg et al., 1990). Invasins of cultured mammalian cells by
the bacterium was demonstrated to require interaction between the
Yersinia invasin molecule and several species of the β1 family of
integrins present on the cultured cells (Tran Van Nhieu et al.,
25   1991) Since T lymphocytes are rich in β1 integrins (especially
activated immune or memory T cells) the effects of invasin on

human T cell have been investigated (Brett et al., 1993). It is
thought that integrins facilitate the migration of immune T cells
out of the blood vessels and through connective tissues to sites
of antigenic challenge through their interaction with
5   extracellular matrix proteins including fibronectin, laminin and
collagen. The carboxy-terminus of the invasin molecule was found
to be co-stimulatory for naive human CD4$^+$ T in the presence of
the non-specific mitogen, anti-CD3 antibody, causing marked
proliferation and expression of cytokines. The specific invasin
10   domain which interacts with the β1 integrins to cause this
stimulation also was identified (Brett et al., 1993). Because of
the demonstrated T cell co-stimulatory properties associated with
this domain, it can be linked to the promiscuous T_h epitope in
the chimeric peptide of the present invention opposite from the B
15   cell epitope.

         Many of the outer membrane proteins of Gram-negative
bacteria are both lipid-modified and very immunogenic. Because
of the apparent correlation between covalent lipid linkage and
immunogenicity, tripalmitoyl-S-glycerol cysteine (Pam$_3$Cys), a
20   lipid common to bacterial membrane proteins, can be coupled to
synthetic peptides representing either B cell or cytotoxic T cell
epitopes. Because significant adjuvanting responses are elicited
by this lipid linkage, promiscuous T_h epitope of the chimeric
peptide can be lipid modified opposite its linkage to the B cell
25   epitope. Such lipid-modified chimeric peptides are likely to be
more immunogenic than the unmodified version of the same peptide.

U.S. Patent 5,843,446, which includes a disclosure of T_h epitopes and the immunostimulatory properties of invasin epitopes and lipid moieties, is herein incorporated entirely by reference.

Immunogenicity can further be significantly improved if the antigens are co-administered with adjuvants. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses, e.g. to vaccines. Intrinsic adjuvants, such as lipopolysaccharides, normally are the components of the killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody

responses to diptheria and tetanus toxoids is well established and a HBsAg vaccine has been adjuvanted with alum as well.

A wide range of extrinsic adjuvants can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes. To efficiently induce humoral immune responses (HIR) and cell-mediated immunity (CMI), immunogens are emulsified in adjuvants. Many adjuvants are toxic, inducing granulomas, acute and chronic inflammations (Freund's complete adjuvant, FCA), cytolysis (saponins and Pluronic polymers) and pyrogenicity, arthritis and anterior uveitis (LPS and MDP). Although FCA is an excellent adjuvant and widely used in research, it is not licensed for use in human or veterinary vaccines because of its toxicity.

U.S. Pat. No. 4,855,283 teaches glycolipid analogues including N-glycosylamides, N-glycosylureas and N-glycosylcarbamates, each of which is substituted in the sugar residue by an amino acid, as immuno-modulators or adjuvants. U.S. Pat. No. 4,258,029 teaches that octadecyl tyrosine hydrochloride (OTH) functions as an adjuvant when complexed with tetanus toxoid and formalin inactived type I, II and III poliomyelitis virus vaccine. Also, Nixon-George et al., 1990, reported that octadecyl esters of aromatic amino acids complexed

with a recombinant hepatitis B surface antigen enhanced the host
immune responses against hepatitis B virus.

The addition of exogenous adjuvant/emulsion
formulations which maximize immune responses to the internal
peptide cleavage product are preferred. The adjuvants and
carriers that are suitable are those: (1) which have been
successfully used in Phase I human trials; (2) based upon their
lack of reactogenicity in preclinical safety studies, have
potential for approval for use in humans; or (3) have been
approved for use in food and companion animals.

Immunotherapy regimens which produce maximal immune
responses following the administration of the fewest number of
doses, ideally only one dose, are highly desirable. This result
can be approached through entrapment of immunogen in
microparticles. For example, the absorbable suture material
poly(lactide-co-glycolide) co-polymer can be fashioned into
microparticles containing immunogen. Following oral or
parenteral administration, microparticle hydrolysis *in vivo*
produces the non-toxic byproducts, lactic and glycolic acids, and
releases immunogen largely unaltered by the entrapment process.
The rate of microparticle degradation and the release of
entrapped immunogen can be controlled by several parameters,
which include (1) the ratio of polymers used in particle
formation (particles with higher co-glycolide concentrations
degrade more rapidly); (2) particle size, (smaller particles
degrade more rapidly than larger ones); and, (3) entrapment

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efficiency, (particles with higher concentrations of entrapped antigen degrade more rapidly than particle with lower loads). Microparticle formulations can also provide primary and subsequent booster immunizations in a single administration by

5   mixing immunogen entrapped microparticles with different release rates. Single dose formulations capable of releasing antigen ranging from less than one week to greater than six months can be readily achieved. Moreover, delivery of the chimeric peptide according to the present invention entrapped in microparticles

10   can also provide improved efficacy when the microparticulate immunogen is mixed with an exogenous adjuvant/emulsion formulations.

The efficacy of the chimeric peptides can be established and analyzed by injecting an animal, e.g., mice or

15   rats, with the chimeric peptide formulated in alum and then following the immune response to the internal peptide cleavage product.

Another aspect of the present invention provides an immunizing composition which includes an immunizing effective

20   amount of one or more of the chimeric peptides of the invention and a pharmaceutically acceptable carrier, excipient, diluent, or auxiliary agent, including adjuvants. Accordingly, the chimeric peptides can be formulated as an immunizing composition using adjuvants, pharmaceutically-acceptable carriers, excipients,

25   diluents, auxiliary agents or other ingredients routinely provided in immunizing compositions. Such formulations are

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readily determined by one of ordinary skill in the art and
include formulations for immediate release and for sustained
release, e.g., microencapsulation. The present immunizing
compositions can be administered by any convenient route
5   including subcutaneous, oral, intramuscular, or other parenteral
or internal route. Similarly the vaccines can be administered as
a single dose or divided into multiple doses for administration.
Immunization schedules are readily determined by the ordinary
skilled artisan. For example, the adjuvants or emulsifiers that
10   can be used in this invention include alum, incomplete Freund's
adjuvant, liposyn, saponin, squalene, L121, emulsigen and ISA720.
In preferred embodiments, the adjuvants/emulsifiers are alum,
incomplete Freund's adjuvant, a combination of liposyn and
saponin, a combination of squalene and L121 or a combination of
15   emulsigned and saponin.

The immunizing compositions of the present invention
contain an immunoeffective amount of one or more of the chimeric
peptides and a pharmaceutically acceptable carrier. Such
compositions in dosage unit form can contain about 0.5 μg to
20   about 1 mg of each peptide per kg body weight. When delivered in
multiple doses, the dosage unit form is conveniently divided into
the appropriate amounts per dosage.

Immunizing compositions which contain cocktails of two
or more of the chimeric peptides of the present invention enhance
25   immunoefficacy in a broader population and thus provide a better
immune response against the internal peptide cleavage product,

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such as amyloid β. Other immunostimulatory synthetic chimeric peptide immunogens are arrived at through modification into lipopeptides so as to provide built-in adjuvanticity for potent vaccines. The immune response to synthetic chimeric peptide immunogens of the present invention can be improved by delivery through entrapment in or on biodegradable microparticles of the type described by O'Hagan et al (1991). The immunogens can be encapsulated with or without adjuvant, including covalently attached lipid moiety such as Pam$_3$Cys, and such microparticles can be administered with an immunostimulatory adjuvant such as Freund's Incomplete Adjuvant or alum. The microparticles function to potentiate immune responses to an immunogen and to provide time-controlled release for sustained or periodic responses. for oral administration, and for topical administration (O'Hagan et al., 1991).

A further aspect of the present invention is a method for immunization against the free N-terminus and/or free C-terminus of a naturally-occurring internal peptide cleavage product, which, when naturally-occurring in a mammal, is derived from a precursor protein or a mature protein. This method according to the present invention involves administering to a mammal an immunizing composition containing the chimeric peptides of the present invention for which the internal peptide cleavage product is a self molecule. A preferred embodiment of this method is one in which the method is directed to immunizing against amyloid β peptides to raise anti-N-terminal or anti-C-

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terminal end-specific amyloid β antibodies which do not cross-
react with the β amyloid precursor protein. In this preferred
embodiment, the N and/or C of formulas (I) and (II) of the
chimeric peptide(s) used in the present method are from the N-
terminus and/or C-terminus of amyloid β peptides.

A still further aspect of the present invention is a
molecule which contains the antigen-binding portion of an
antibody, preferably a monoclonal antibody, specific for the
chimeric peptide according to the present invention. This
molecule can be an antibody raised against the chimeric peptide
in vivo or can be a recombinant antibody that is intended to
encompass fragments of antibodies containing the antigen-binding
portion, chimeric or humanized immunoglobulin molecules of any
isotype, as well as a single-chain antibodies.

Chimeric antibodies are understood to be molecules,
different portions of which are derived from different animal
species, such as those humanized antibodies having a variable
region derived from a mouse monoclonal antibody and a human
immunoglobulin constant region. Chimeric antibodies and methods
for their production are well known in the art. For example, the
DNA encoding the variable region of the antibody can be inserted
into or joined with DNA encoding other antibodies to produce
chimeric antibodies (U.S. patent 4,816,567; Orlandi et al.,
1989).

Single-chain antibodies can be single chain composite
polypeptides having end-specific peptide binding capability and

comprising a pair of amino acid sequences homologous or analogous to the variable regions of an immunoglobulin light and heavy chain (linked V_H-V_L or single chain Fv). Both V_H and V_L may copy natural monoclonal antibody sequences, or one or both of the chains may comprise a CDR-FR construct of the type described in U.S. Patent 5,091,513. The separate polypeptides analogous to the variable regions of the light and heavy chains are held together by a peptide linker. Methods of production of such single chain antibodies, e.g., single chain Fv (scFv), particularly where the DNA encoding the polypeptide structures of the V_H and V_L chains are characterized or can be readily ascertained by sequence analysis, may be accomplished in accordance with the methods described, for example, in U.S. Patent 4,946,778, U.S. Patent 5,091,513, U.S. Patent 5,096,815, Biocca et al. (1993), Duan et al. (1994), Mhashilkar et al., (1995), Marasco et al. (1993), and Richardson et al. (1995).

Besides the conventional method of raising antibodies *in vivo*, antibodies can be produced *in vitro* using Phage Display technology. The production of recombinant antibodies is much faster compared to conventional antibody production and they can be generated against an enormous number of antigens. In contrast, in the conventional method, many antigens prove to be non-immunogenic or extremely toxic, and therefore cannot be used to generate antibodies in animals. Moreover, affinity maturation (i.e., increasing the affinity and specificity) of recombinant antibodies is very simple and relatively fast. Finally, large

numbers of different antibodies against a specific antigen can be generated in one selection procedure. To generate recombinant monoclonal antibodies one can use various methods all based on Phage Display libraries to generate a large pool of antibodies

5   with different antigen recognition sites. Such a library can be made in several ways: One can generate a synthetic repertoire by cloning synthetic CDR3 regions in a pool of heavy chain germline genes and thus generating a large antibody repertoire, from which recombinant antibody fragments with various specificities can be

10 selected. One can use the lymphocyte pool of humans as starting material for the construction of an antibody library. It is possible to construct naive repertoires of human IgM antibodies and thus create a human library of large diversity. This method has been widely used successfully to select a large number of

15 antibodies against different antigens.

Yet another aspect of the present invention is a method for passive immunization involving administering to a mammal, preferably human, the molecule which contains the antigen-binding portion of an antibody specific for the chimeric peptide

20 according to the present invention for which the internal peptide cleavage product is a self molecule. A preferred embodiment of this method is one in which the method is directed to passively immunizing with molecules in which the antigen-binding portion thereof is end-specific for amyloid β peptides.

25 Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be

performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection
5   with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come
10 within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journal articles
15 or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the
20 references cited within the references cited herein are also entirely incorporated by references.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the
25 present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily

5   modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments,

10   based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and

15   guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

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